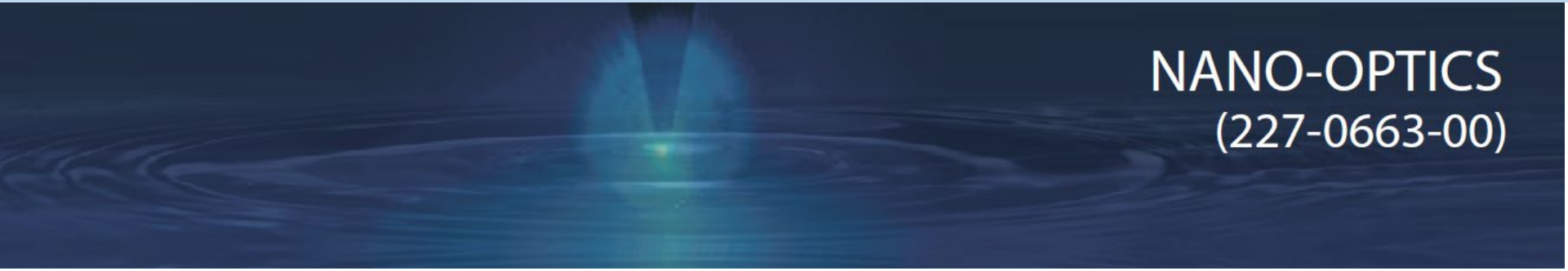
Welcome again!



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Photonics Laboratory
HPP M24

- Get in touch for MSc projects!
- Who is interested in a lab tour?
- Did everyone settle in with their literature group?
- What's going on with the homeworks?

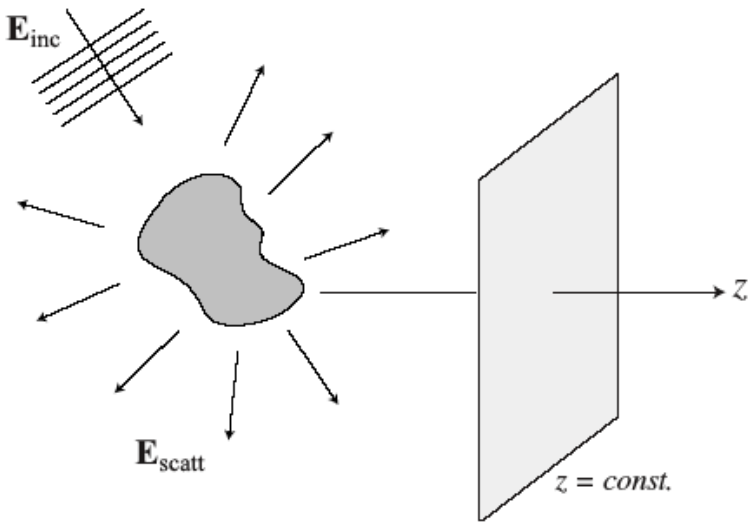
Today's question

Why do I not see the atoms that make up your skin when I look at you?

On the menu today

- Motivation: Why nano-optics?
- Repetition: electromagnetism
- Optical imaging:
  - Description of a focused field
    - Gaussian beam (paraxial approximation)
    - Method of Richards and Wolf
  - The diffraction limit
  - Fluorophores
  - Example: Fluorescence microscopy
  - Example: STED microscopy
  - Example: Localization microscopy
  - Example: Scanning probe microscopy

Angular spectrum



MATH :

$$\hat{\mathbf{E}}(k_x, k_y;\, z) \;=\; \frac{1}{4\pi^2} \iint_{-\infty}^{\infty} \mathbf{E}(x, y, z)\, \mathrm{e}^{-i\,[k_x\, x + k_y\, y]}\; dx\, dy$$

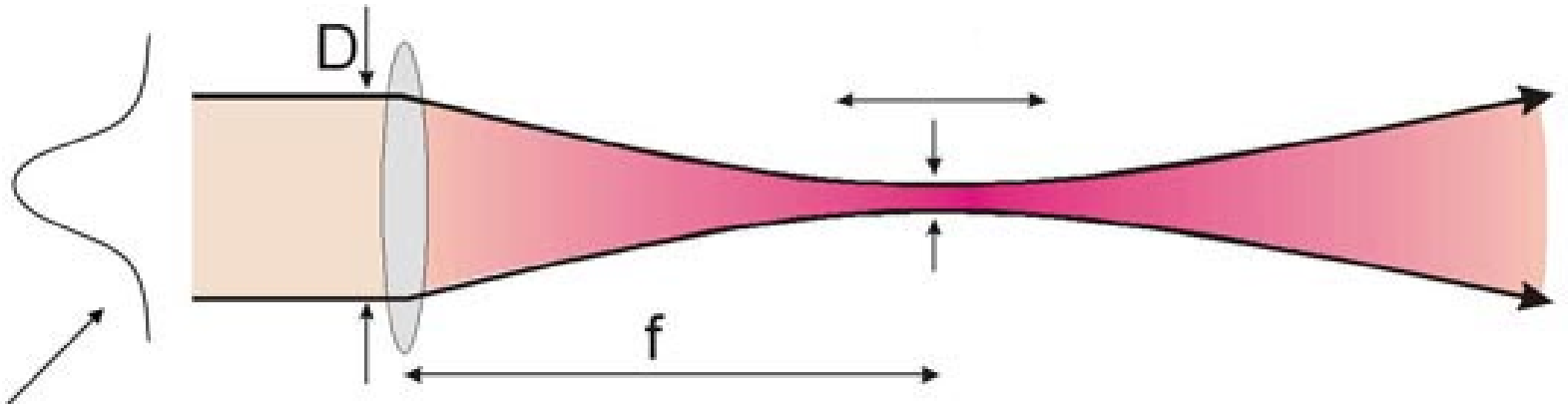
$$\mathbf{E}(x, y, z) \;=\; \iint_{-\infty}^{\infty} \hat{\mathbf{E}}(k_x, k_y;\, z)\, \mathrm{e}^{i\,[k_x\, x + k_y\, y]}\; dk_x\, dk_y$$

PHYS : $\quad (\nabla^2 + k^2)\, \mathbf{E}(\mathbf{r}) \;=\; 0 \quad \longrightarrow \quad \hat{\mathbf{E}}(k_x, k_y;\, z) \;=\; \hat{\mathbf{E}}(k_x, k_y;\, 0)\, \mathrm{e}^{\pm i\, k_z\, z}$

Together:

$$\mathbf{E}(x, y, z) \;=\; \iint_{-\infty}^{\infty} \hat{\mathbf{E}}(k_x, k_y;\, 0)\, \mathrm{e}^{i\,[k_x\, x + k_y\, y \pm k_z\, z]}\; \mathrm{d}k_x\, \mathrm{d}k_y$$

The Gaussian Beam



Field in focal plane z=0: $$\mathbf{E}(x', y', 0) = \mathbf{E}_o \, \mathrm{e}^{-(x'^2+y'^2)/w_0^2}$$

$$\mathbf{E}(\rho, z) = \mathbf{E}_o \frac{w_o}{w(z)} \, \mathrm{e}^{-\frac{\rho^2}{w^2(z)}} \, \mathrm{e}^{i\,[kz - \eta(z) + k\rho^2/2R(z)]}$$

$$w(z) = w_o \, (1 + z^2/z_o^2)^{1/2}$$ Beam waist

$$R(z) = z \, (1 + z_o^2/z^2)$$ Wavefront radius

$$\eta(z) = \arctan z/z_o$$ Phase correction (Gouy phase)

$$z_o = \frac{k \, w_o^2}{2} \, ;$$ Rayleigh length

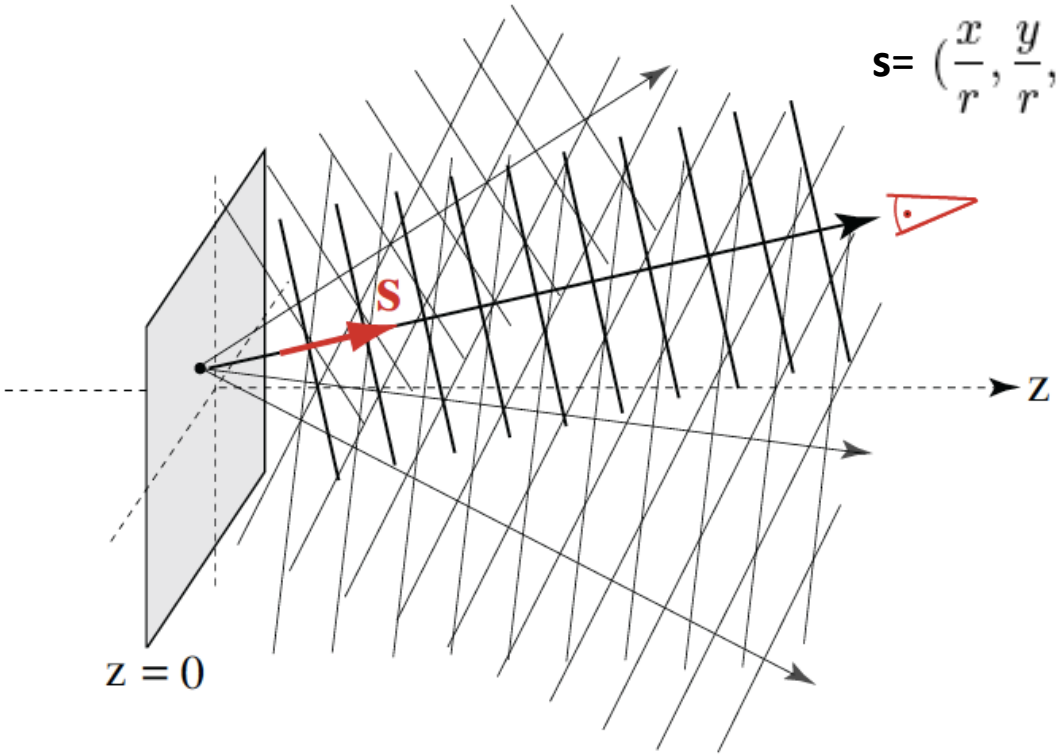
The Gaussian Beam

$$z_o = \frac{k\,w_o^2}{2} \qquad \theta = \frac{2}{k\,w_o}$$

The Gaussian Beam has one free parameter. Which one?

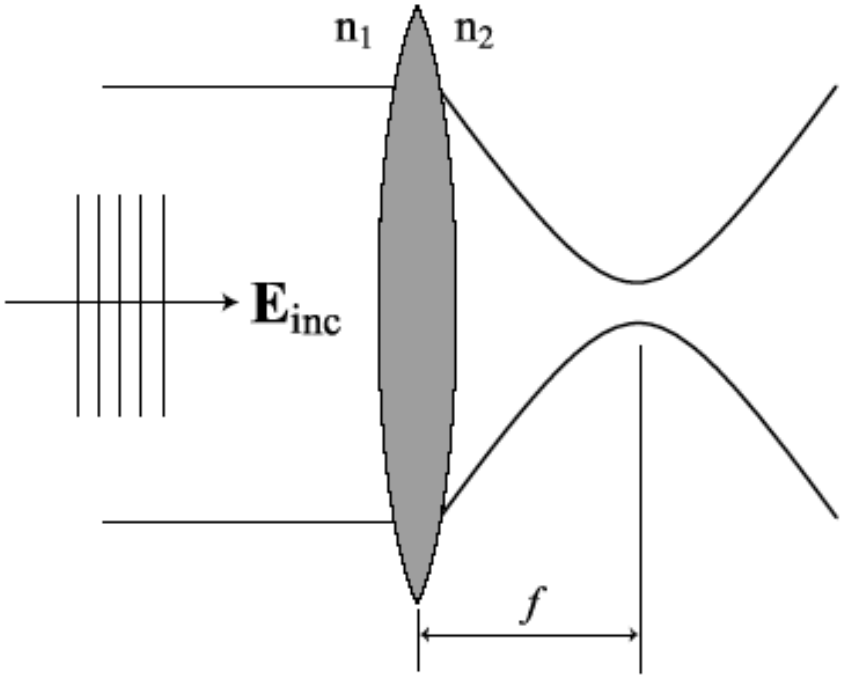
Far-field

$$\mathbf{E}_\infty(s_x, s_y) \;=\; -2\pi \mathrm{i} k\, s_z\, \hat{\mathbf{E}}(k s_x, k s_y;\, 0)\, \frac{\mathrm{e}^{\mathrm{i}kr}}{r}$$



A better description of focused fields

Better than Gaussian beams, but not much to do analytically.
Methods of Richards and Wolf; see also “Principles of Nano Optics”



Angular spectrum in terms of far-field

$$\mathbf{E}(x,y,z) = \iint_{-\infty}^{\infty} \underbrace{\hat{\mathbf{E}}(k_x,k_y;0)}\, \mathrm{e}^{\mathrm{i}\,[k_x x + k_y y \pm k_z z]}\, \mathrm{d}k_x\, \mathrm{d}k_y$$

From method of stationary phase: $= \dfrac{ir\,\mathrm{e}^{-ikr}}{2\pi k_z}\, \mathbf{E}_\infty(k_x,k_y)$

$$\mathbf{E}(x,y,z) = \frac{\mathrm{i}r\,\mathrm{e}^{-\mathrm{i}kr}}{2\pi} \iint_{(k_x^2+k_y^2)\le k^2} \mathbf{E}_\infty\left(\frac{k_x}{k},\frac{k_y}{k}\right)\, \mathrm{e}^{\mathrm{i}\,[k_x x + k_y y \pm k_z z]}\, \frac{1}{k_z}\, \mathrm{d}k_x\, \mathrm{d}k_y$$

$$k_x \to k s_x$$
$$k_y \to k s_y$$

For $k_z \sim k$: Fourier Optics !

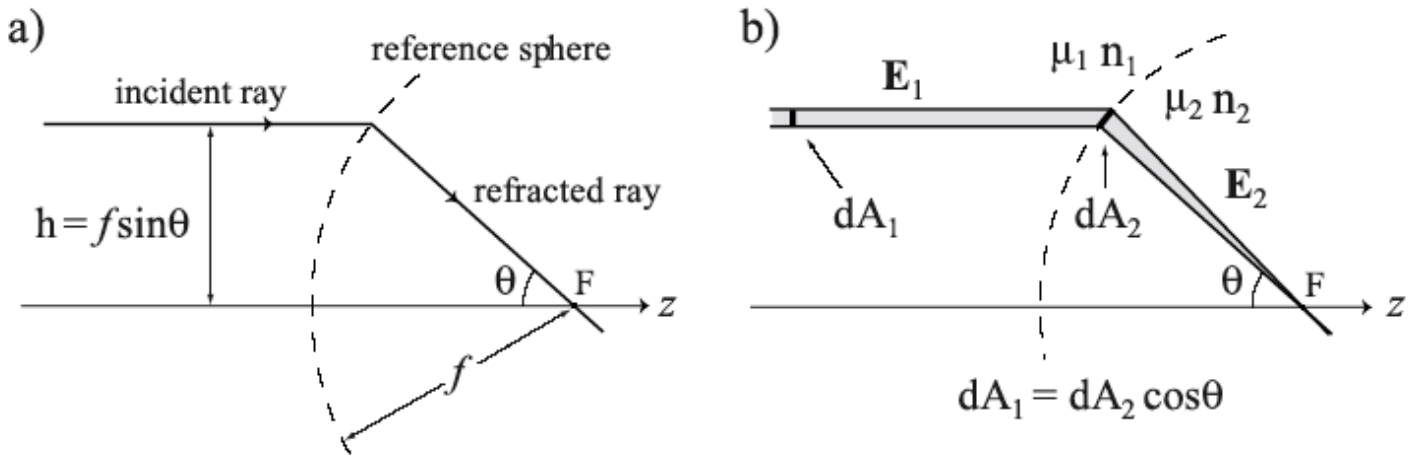
Back to the lens

- We can calculate the field near a focus if we just know the far-field


Boundless.com

$$\mathbf{E}(x,y,z) = \frac{\mathrm{i}r\,\mathrm{e}^{-\mathrm{i}kr}}{2\pi} \iint_{(k_x^2+k_y^2)\leq k^2} \mathbf{E}_\infty\left(\frac{k_x}{k},\frac{k_y}{k}\right)\,\mathrm{e}^{\mathrm{i}[k_x x + k_y y \pm k_z z]}\,\frac{1}{k_z}\,\mathrm{d}k_x\,\mathrm{d}k_y$$

So what does a lens do?



$$\mathbf{E}(x,y,z) = \frac{\mathrm{i}r\,\mathrm{e}^{-\mathrm{i}kr}}{2\pi} \iint_{(k_x^2+k_y^2)\le k^2} \mathbf{E}_\infty\left(\frac{k_x}{k},\frac{k_y}{k}\right)\,\mathrm{e}^{\mathrm{i}[k_x x + k_y y \pm k_z z]}\,\frac{1}{k_z}\,\mathrm{d}k_x\,\mathrm{d}k_y$$

The solution… is a bit lengthy

$(0,0)$ *mode* :

$$\mathbf{E}(\rho,\varphi,z) = \frac{ikf}{2}\sqrt{\tfrac{n_1}{n_2}}\,E_o\,\mathrm{e}^{-ikf}\begin{bmatrix} I_{00}+I_{02}\cos 2\varphi \\ I_{02}\sin 2\varphi \\ -2iI_{01}\cos\varphi \end{bmatrix}$$

$$\mathbf{H}(\rho,\varphi,z) = \frac{ikf}{2Z_{\mu\varepsilon}}\sqrt{\tfrac{n_1}{n_2}}\,E_o\,\mathrm{e}^{-ikf}\begin{bmatrix} I_{02}\sin 2\varphi \\ I_{00}-I_{02}\cos 2\varphi \\ -2iI_{01}\sin\varphi \end{bmatrix}$$

Apodization function:

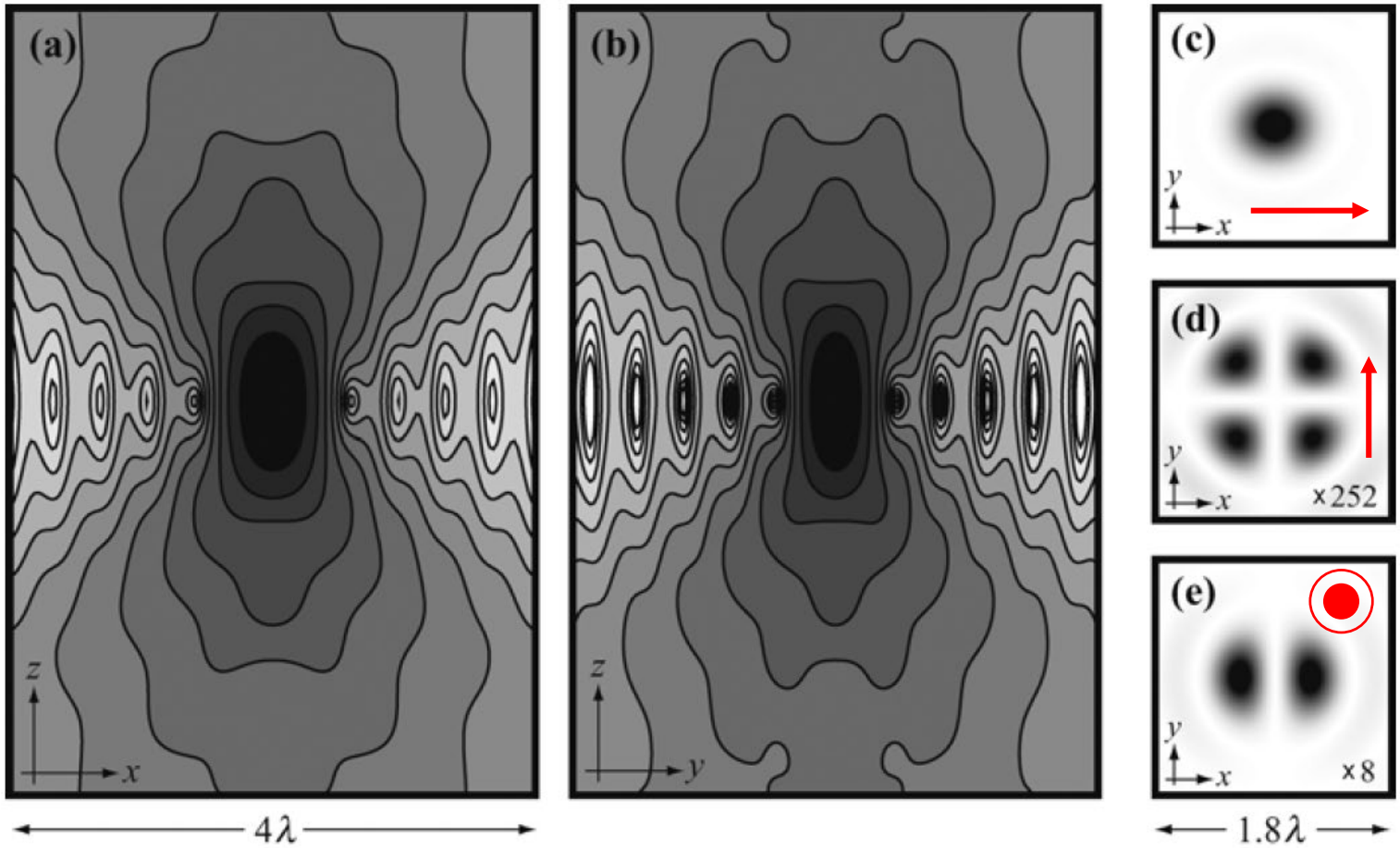
$$f_w(\theta) = \mathrm{e}^{-\frac{1}{f_o^2}\frac{\sin^2\theta}{\sin^2\theta_{max}}}$$

$$I_{00} = \int_0^{\theta_{max}} f_w(\theta)\,(\cos\theta)^{1/2}\sin\theta\,(1+\cos\theta)\,J_0(k\rho\sin\theta)\,\mathrm{e}^{ikz\cos\theta}\,d\theta$$

$$I_{01} = \int_0^{\theta_{max}} f_w(\theta)\,(\cos\theta)^{1/2}\sin^2\theta\,J_1(k\rho\sin\theta)\,\mathrm{e}^{ikz\cos\theta}\,d\theta$$

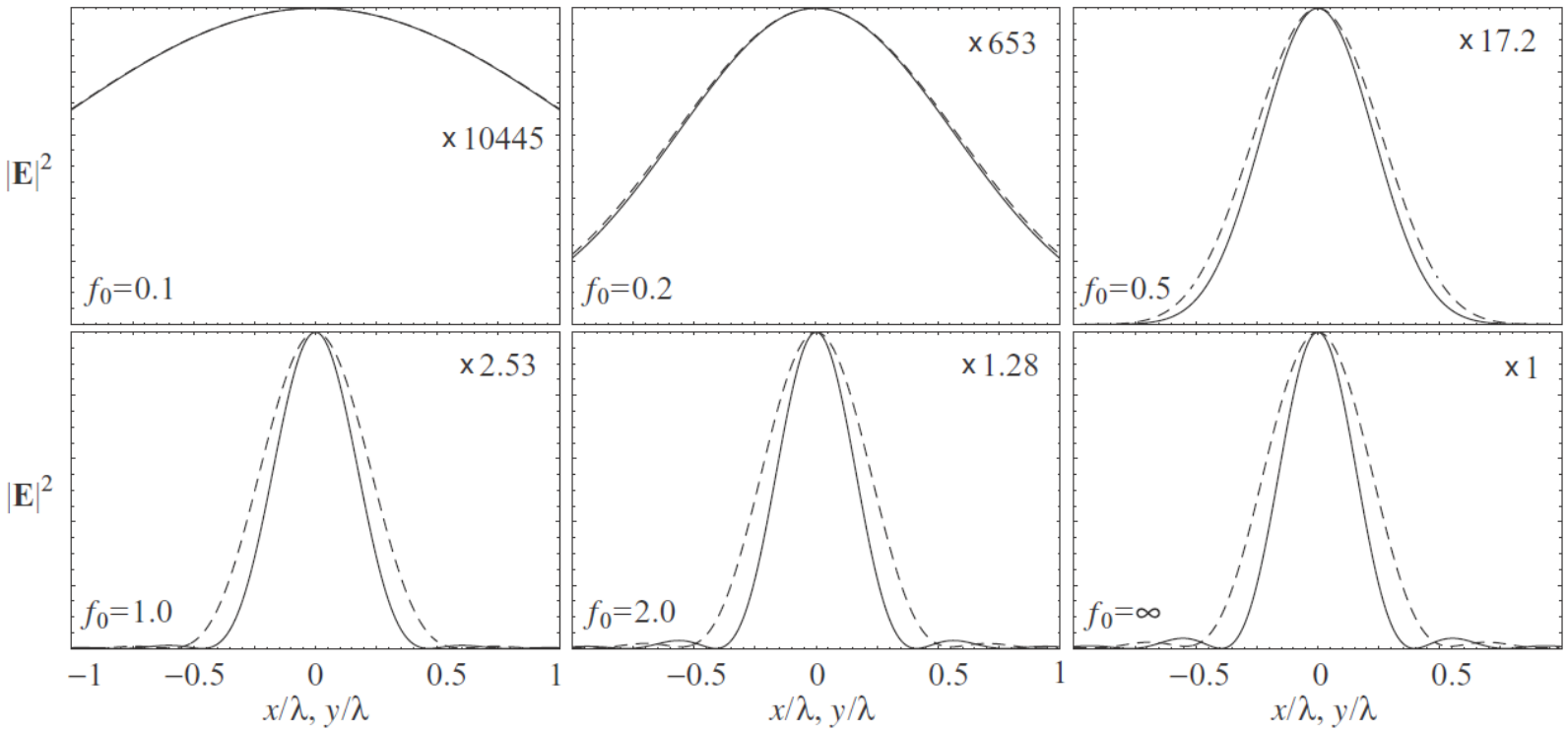
$$I_{02} = \int_0^{\theta_{max}} f_w(\theta)\,(\cos\theta)^{1/2}\sin\theta\,(1-\cos\theta)\,J_2(k\rho\sin\theta)\,\mathrm{e}^{ikz\cos\theta}\,d\theta$$

Strongly focused Gaussian beam



Contour plots of constant $|\boldsymbol{E}|^2$ in the focal region of a focused Gaussian beam (NA = 1.4, n = 1.518, f_0 = 1): (a) in the plane of incident polarization (x, z); (b) in the plane perpendicular to the plane of incident polarization (y, z). A logarithmic scaling is used, with a factor of 2 difference between adjacent contour lines. Images (c), (d), and (e) show the magnitudes of the individual field components $|\boldsymbol{E}_x|^2$, $|\boldsymbol{E}_y|^2$, and $|\boldsymbol{E}_z|^2$, respectively, in the focal plane (z = 0).

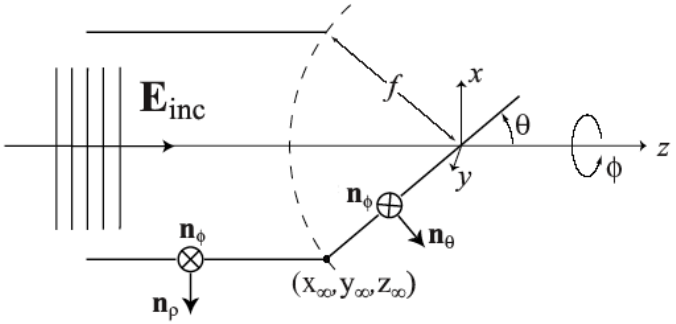
Strongly focused Gaussian beam



Influence of the filling factor f_0 of the back-aperture on the sharpness of the focus. A lens with NA = 1.4 is assumed and the index of refraction is 1.518. The figure shows the magnitude of the electric field intensity $|\boldsymbol{E}|^2$ in the focal plane $z = 0$. The dashed curves have been evaluated along the x-direction (plane of polarization) and the solid curves along the y-direction. All curves have been scaled to an equal amplitude. The scaling factor is indicated in the figures. The larger the filling factor, the bigger the deviation between the solid and dashed curves, indicating the importance of polarization effects.

Weakly focused beam

- Assume strongly overfilled back-aperture
- Assume small NA



$$\mathbf{E}_{inc} = E_{inc}\,\mathbf{n}_x \qquad t_\theta^s = t_\theta^p = 1 \qquad :$$

Focal plane (z=0):

$$I_{00} \approx \frac{2}{k\rho} \int_0^{k\rho\theta_{max}} x\,J_0(x)\,dx = 2\theta_{max}^2 \frac{J_1(k\rho\theta_{max})}{k\rho\theta_{max}}$$

$$\mathbf{E} \approx ikf\,\theta_{max}^2\,E_o\,e^{-ikf}\,\frac{J_1(k\rho\theta_{max})}{k\rho\theta_{max}}\,\mathbf{n}_x$$

Not Gaussian !

Why is this a jinc?

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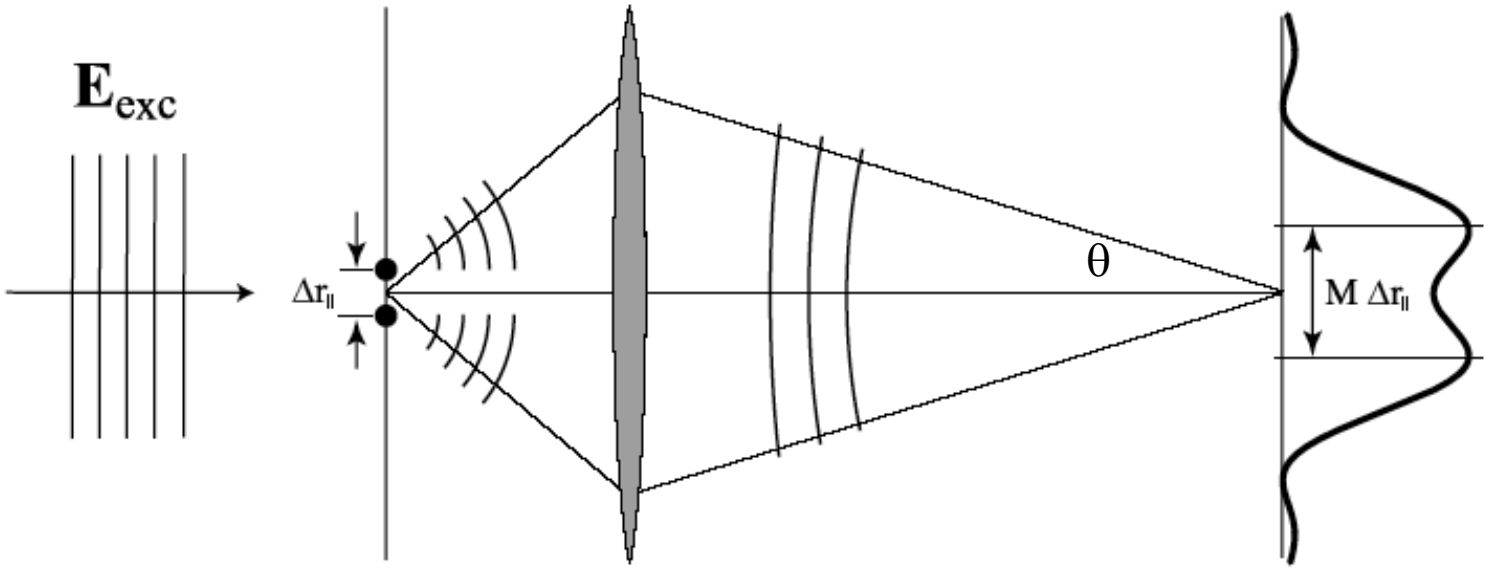
Classical resolution limit



$$\Delta k_{||}\,\Delta r_{||} \geq 1 \qquad \mathrm{Min}\,[\Delta r_{||}] = \frac{\lambda}{4\pi n} \qquad \mathrm{Min}\,[\Delta r_{||}] = \frac{\lambda}{4\pi NA}$$

$$\mathrm{Abbe\,(1873):} \qquad \mathrm{Min}\,[\Delta r_{||}] = 0.6098\,\frac{\lambda}{NA}$$

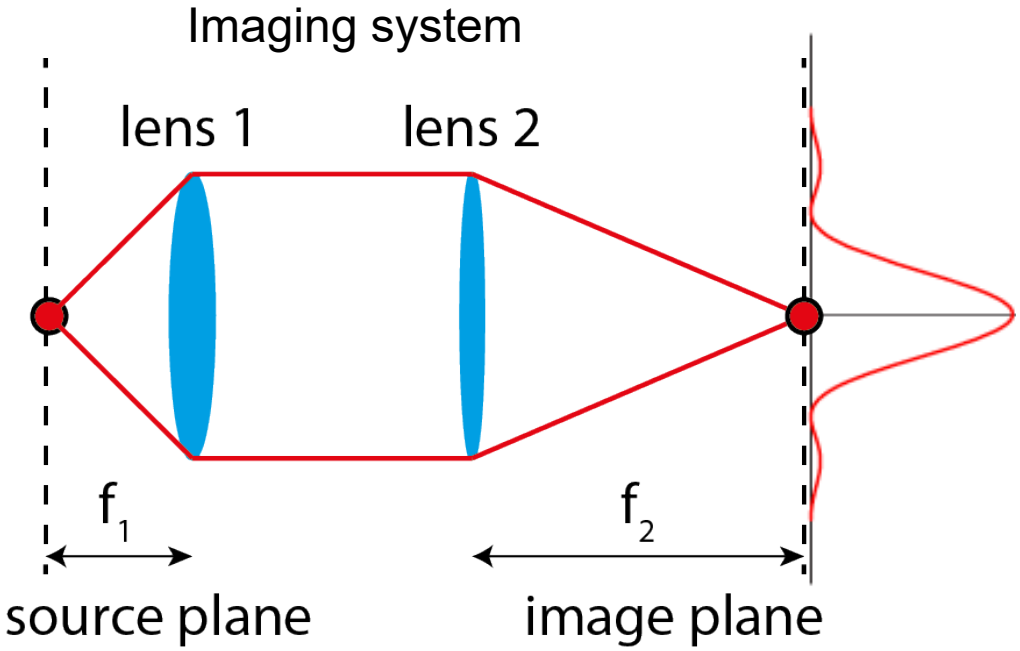
NA = n sinθ

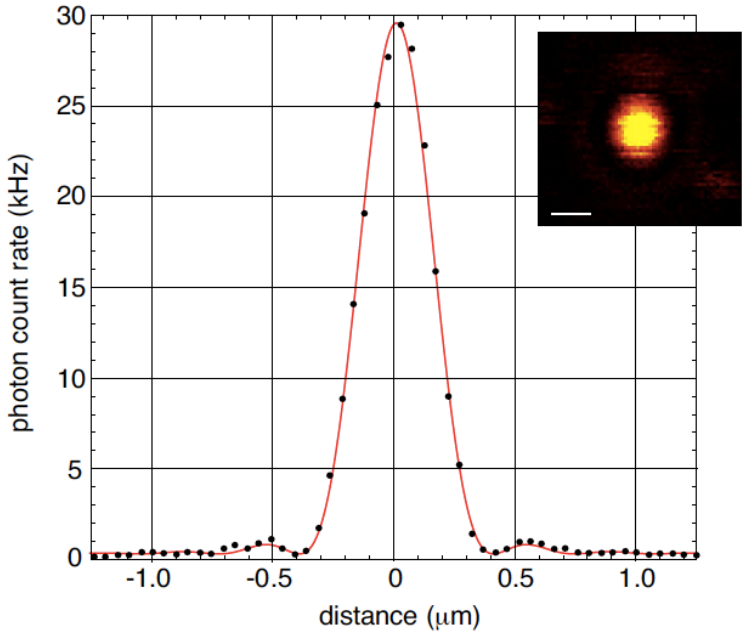
E. Abbe, Arch. Mikrosk. Anat. **9**, 413 (1873).

Abbe's Resolution Limit

E. Abbe, Arch. Mikrosk. Anat. **9**, 413 (1873).

Point-spread function (PSF)





- The PSF is the image of a (mathematical) point source
- The PSF is not a point but spread to a width … because …

Where do you get a mathematical point source from?

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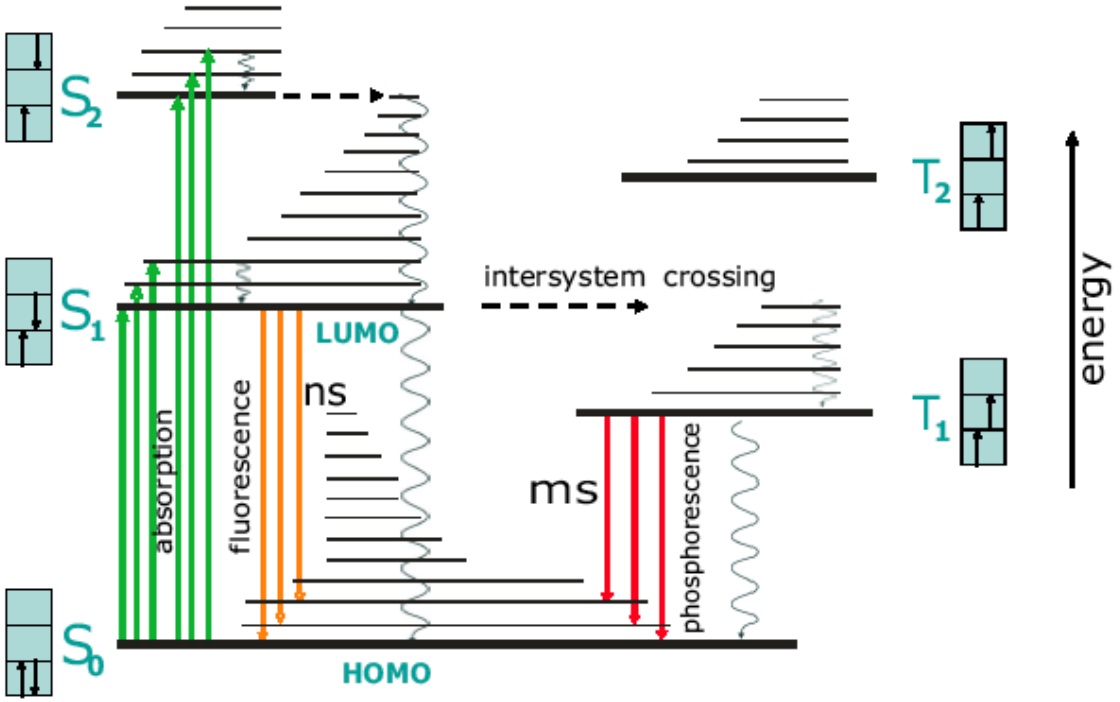
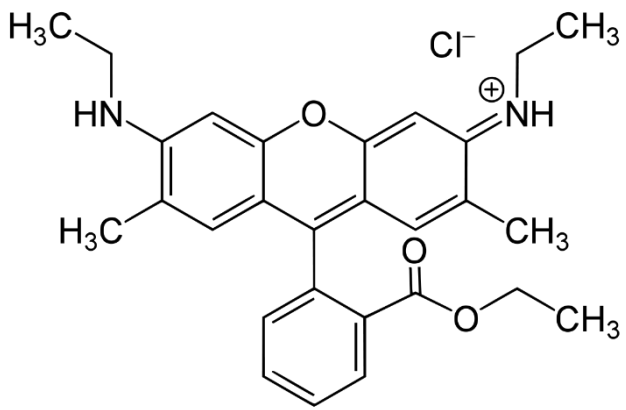
Fluorescent molecules – Jablonski diagram



Rhodamine 6G

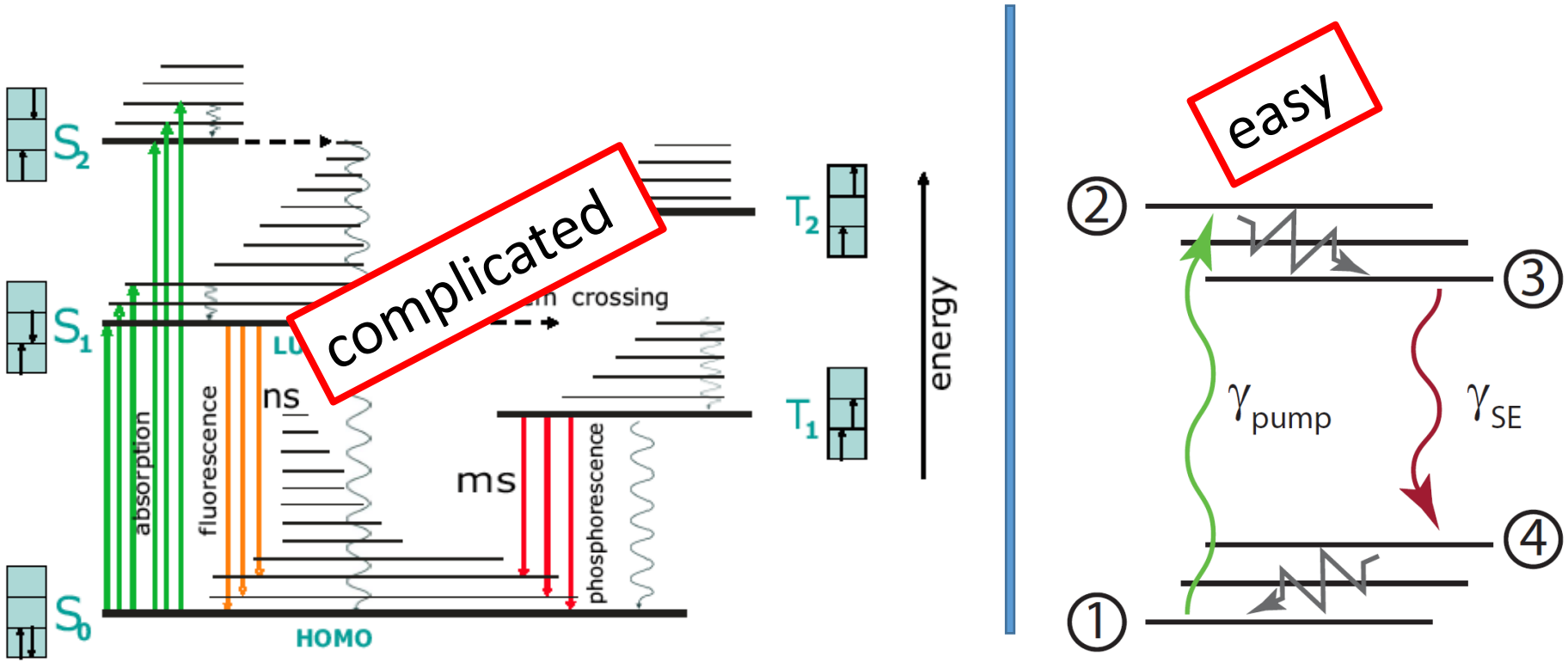


- Stokes shift of fluorescence allows to spectrally separate (intense) pump light from (weak) fluorescence

$$\text{Excitation rate} \sim |\boldsymbol{\mu}\cdot\mathbf{E}(x,y;z_o)|^2$$

μ: transition dipole moment

Fluorescent molecules – Jablonski diagram



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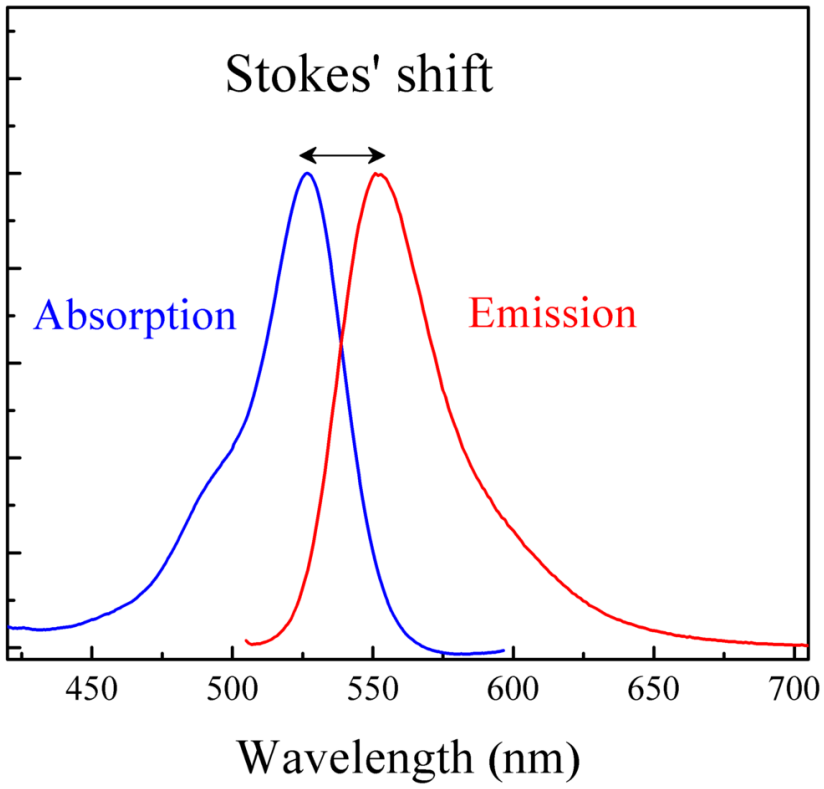

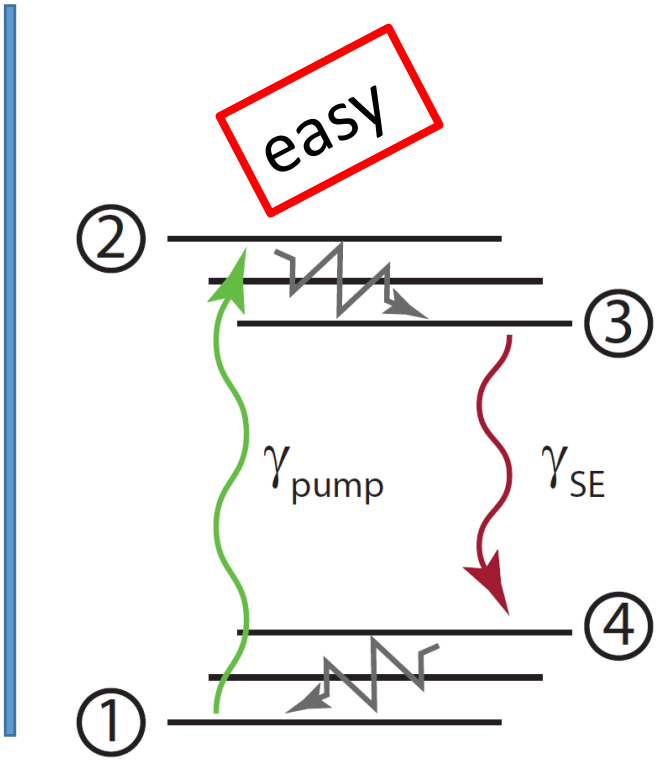

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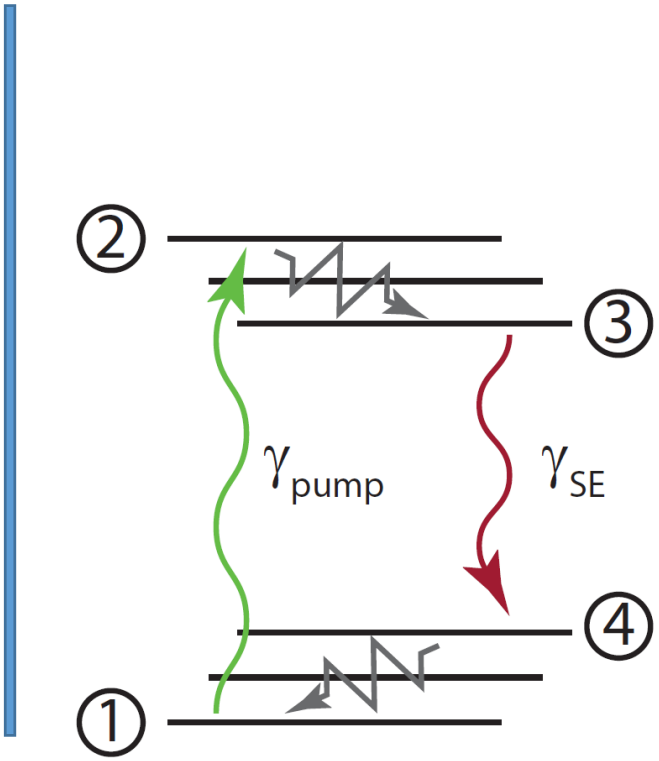
Fluorescent molecules – Jablonski diagram

- In practice, we often quantify the interaction rate between a fluorophore and a light field via a cross section σ

$$\gamma = \frac{|\mathbf{S}|}{\hbar\omega}\sigma$$



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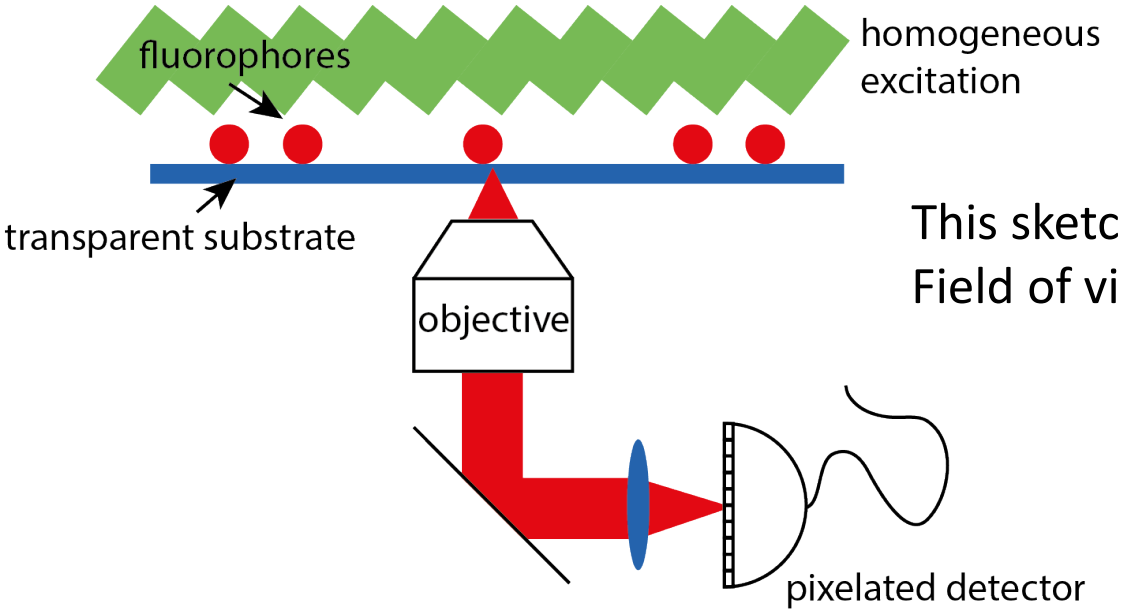
Fluorescence microscopy: Epi-illumination

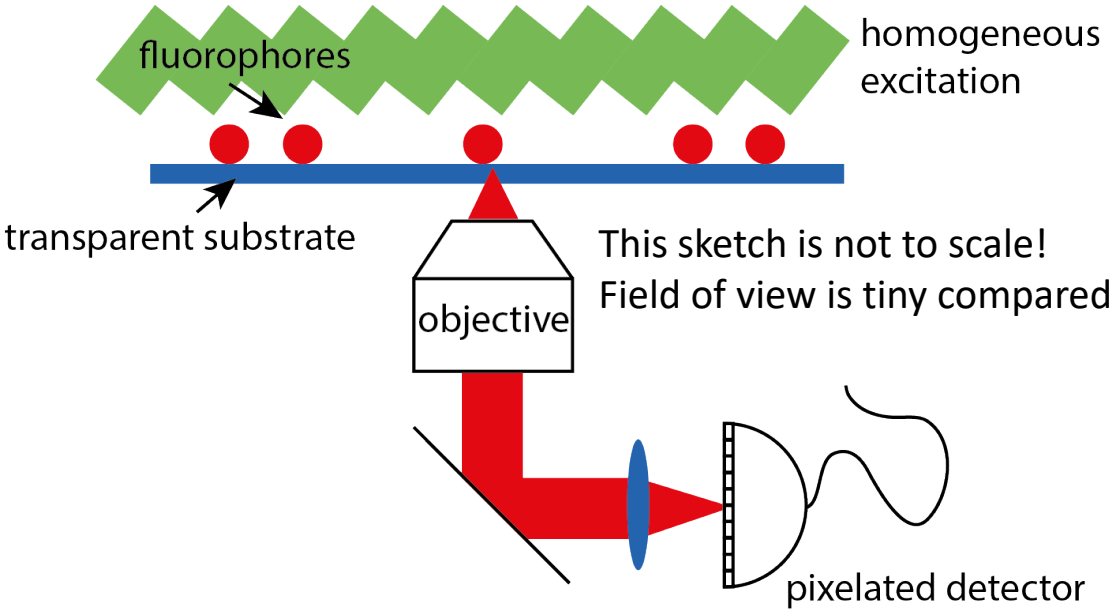


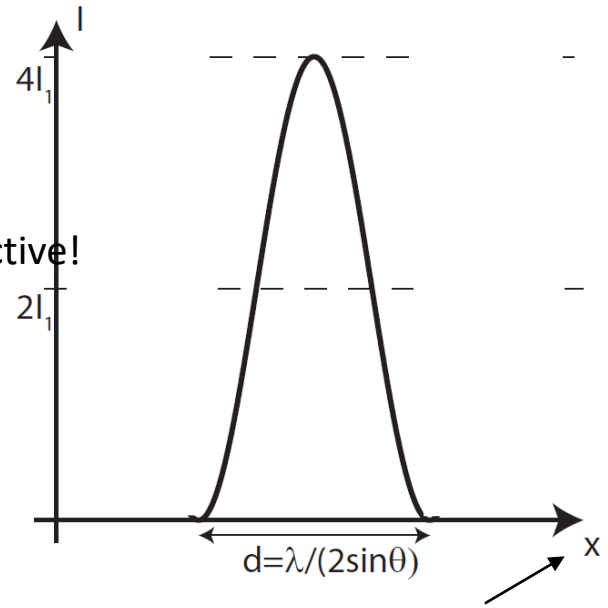
This sketch is not to scale!
Field of view is tiny compared to objective!

- Illuminate entire sample homogeneously
- Image sample plane onto pixelated detector
- Each fluorophore generates a signal according to the PSF
- Resolution is

$$x_0 = \frac{\lambda}{2\,\mathrm{NA}}$$

Fluorescence microscopy: Epi-illumination



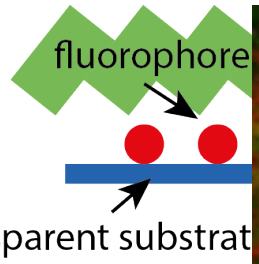


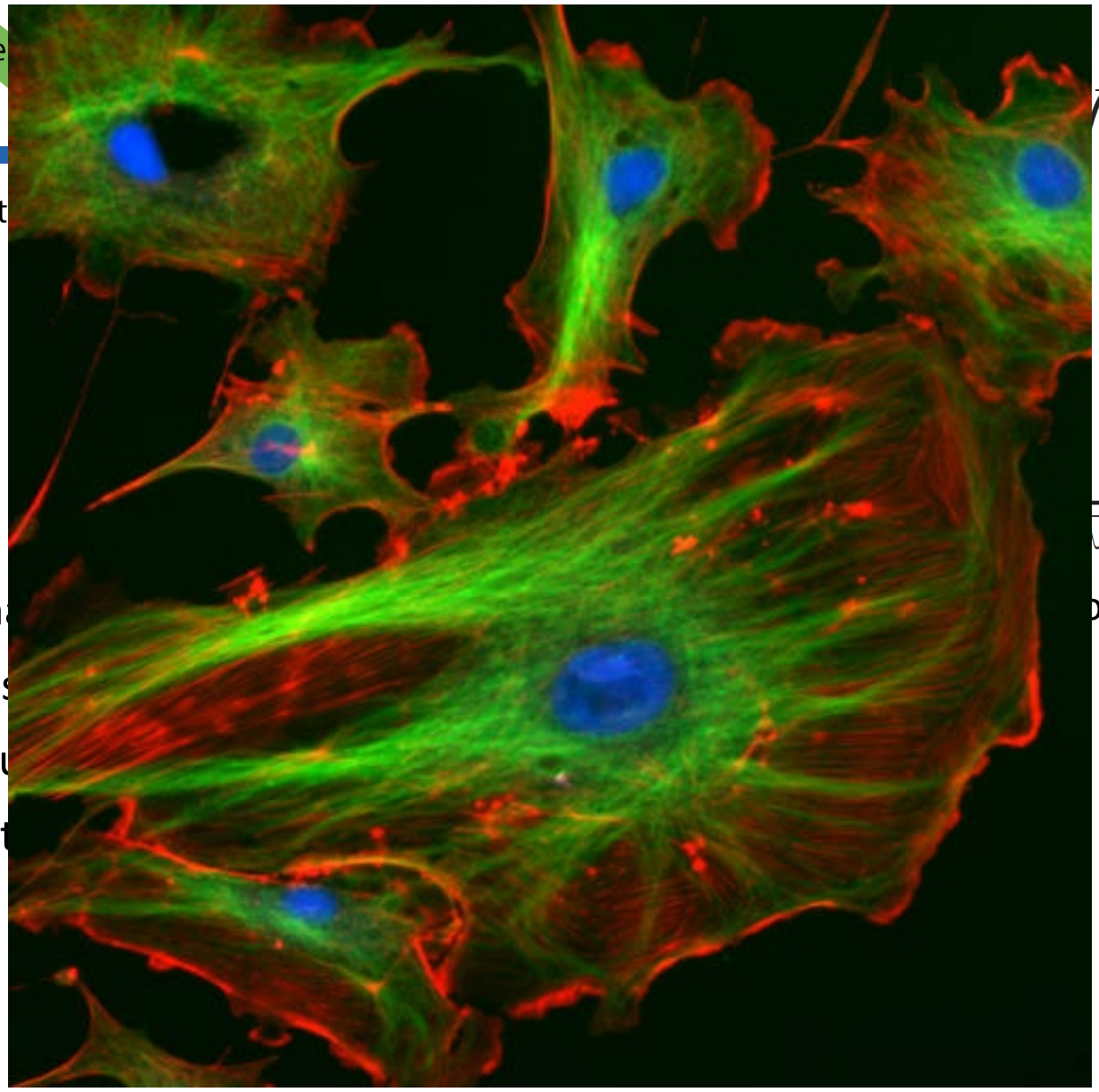
- Illuminate entire sample homogeneously
- Image sample plane onto pixelated detector
- Each fluorophore generates a signal according to the PSF
- Resolution is

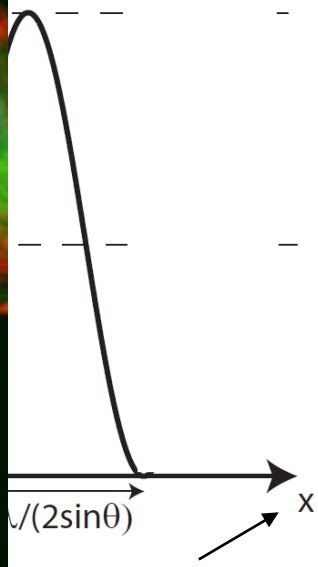
$$x_0 = \frac{\lambda}{2\,\mathrm{NA}}$$

A real microscope … is no different

Fluorescence microscopy: Epi-illumination



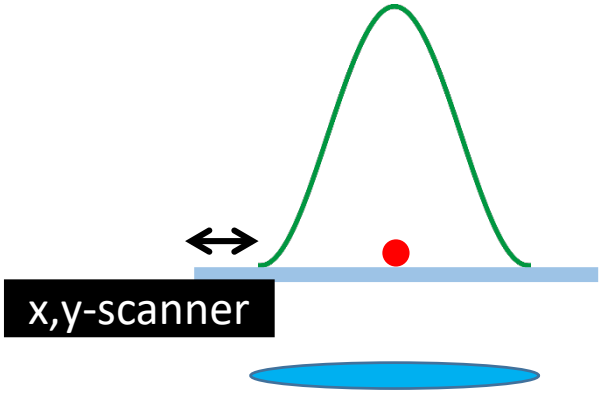


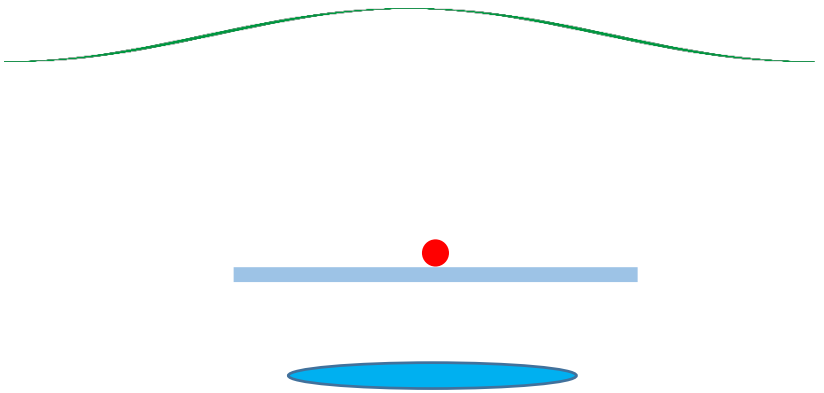


- Illumin
- Image s
- Each flu
- Resolut

on on detector

Fluorescence microscopy – scanning vs. wide-field





Scanning technique.

Resolution s

pump spot on sample

(no spatial resolution)

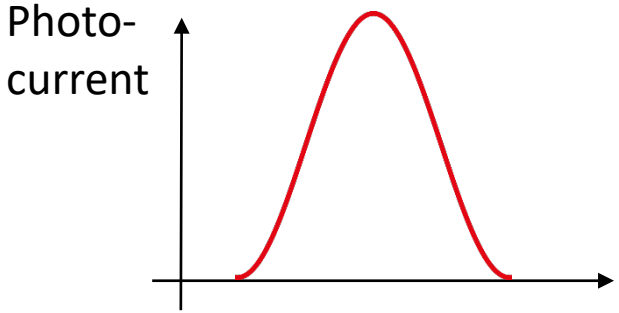
Both limited by diffraction.

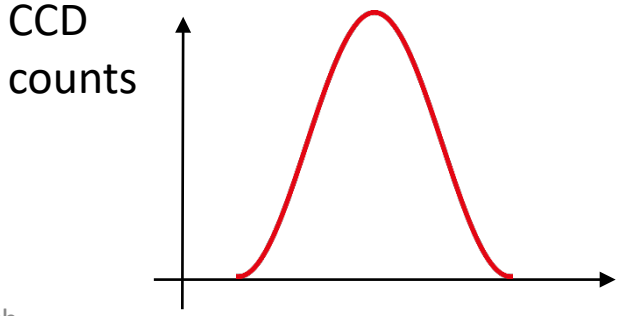
Wide-field imaging.

t by PSF of

imaging system

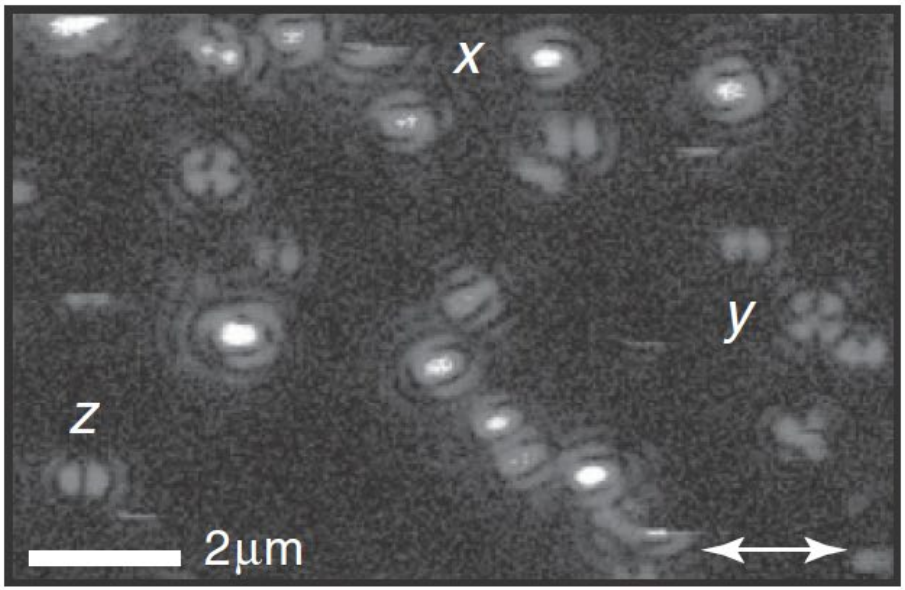
CCD camera

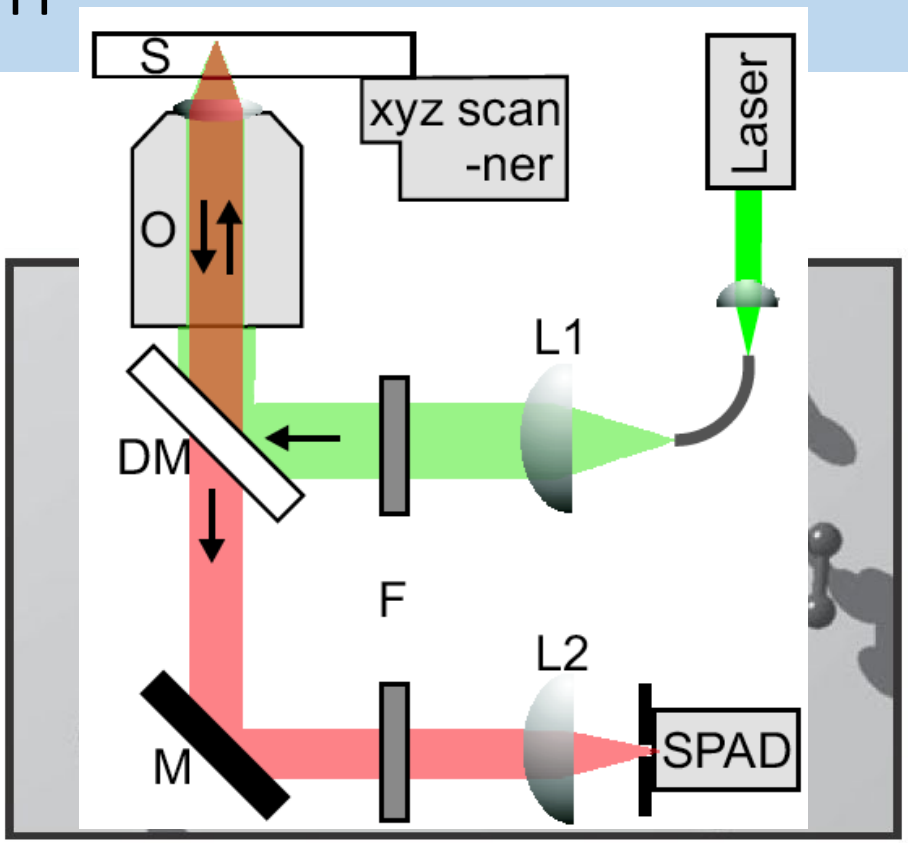


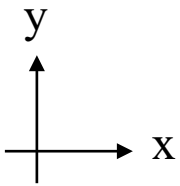


Single molecule detection

Sample: molecules dispersed on glass



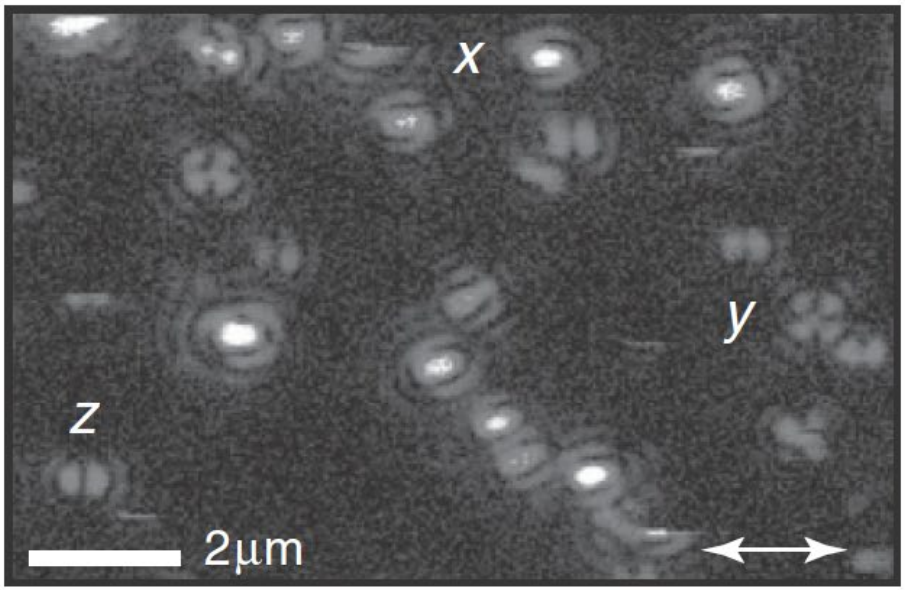


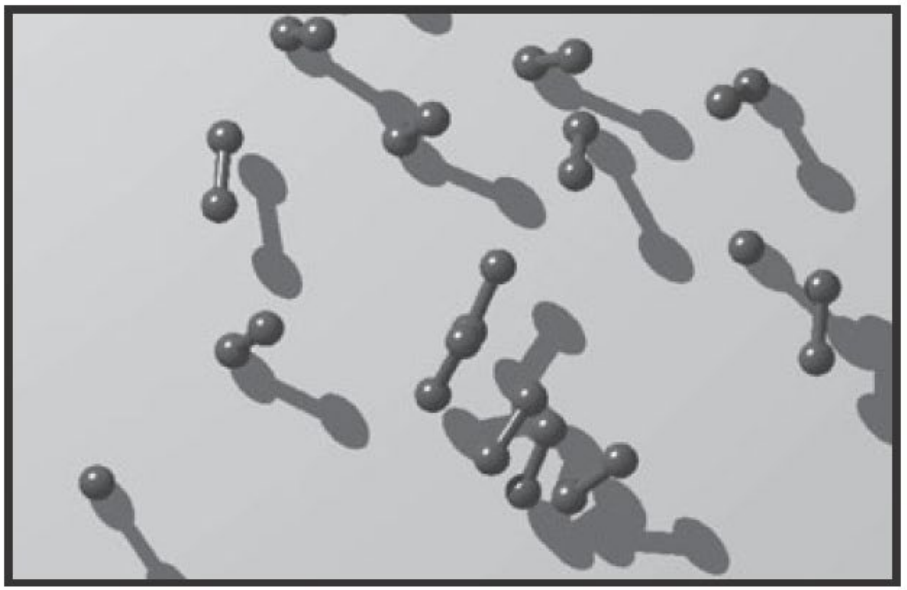


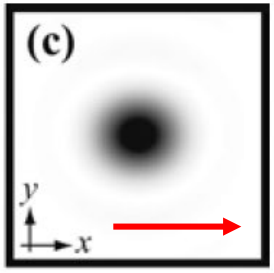
fluorescence rate ~ excitation rate

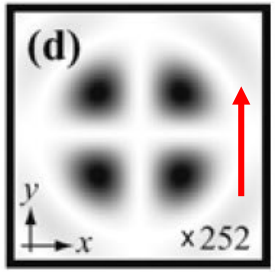
$$\text{contrast} \sim |\boldsymbol{\mu}\cdot\mathbf{E}(x,y;z_o)|^2$$

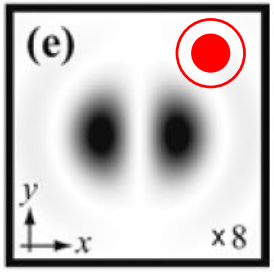
Single molecule detection





Single-molecule excitation 





ed in the focal plane of a strongly focused laser bean ed in the color scale. The excitation rate in each pixel l vector and the molecular absorption dipole moment ipole moments to be reconstructed from the recorded patterns. Compare the patterns marked *x*, *y*, and *z* with those in Fig. 3.11.

STED microscopy

Westphal and Hell, Phys. Rev. Lett. 94, 143903 (2005)

- STED = stimulated emission depletion
- Allows fluorescence microscopy beyond the diffraction limit
- Ingredients:
  - (at least) 4-level system
  - Pump laser
  - Depletion laser

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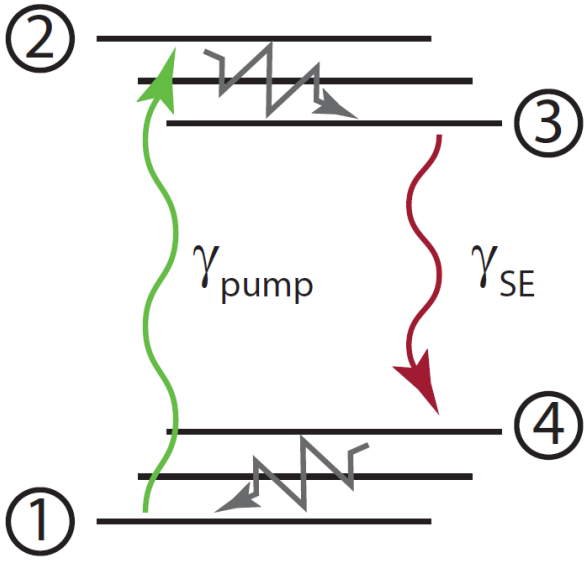
- We need to understand
  - The diffraction limit
  - A four-level system in the presence of light fields

Population of excited state in absence of STED beam

- 4-level system created by two electronic states (of a fluorophore) and vibrational excitation
- Vibrational relaxation infinitely fast
- Start in ground state, turn on pump



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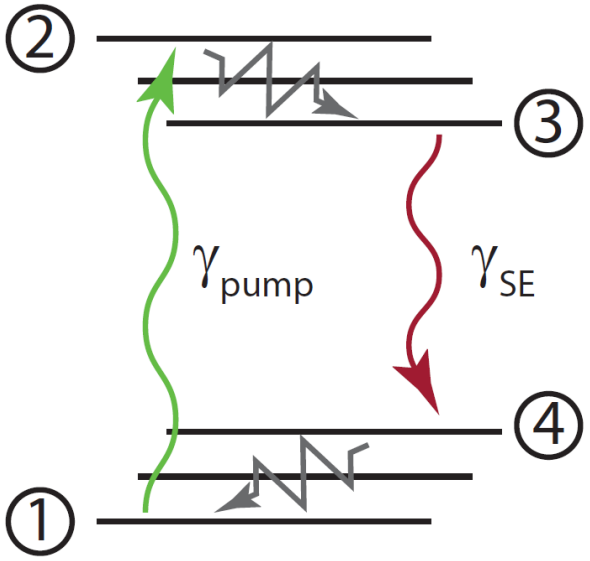

$$p_3(t) = \frac{\gamma_{\text{pump}}}{\gamma_{\text{pump}} + \gamma_{\text{SE}}} \left(1 - \mathrm{e}^{-(\gamma_{\text{pump}} + \gamma_{\text{SE}})t}\right)$$

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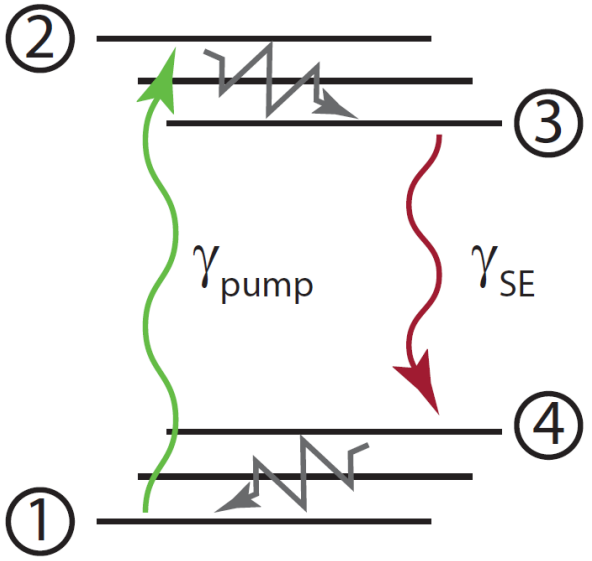
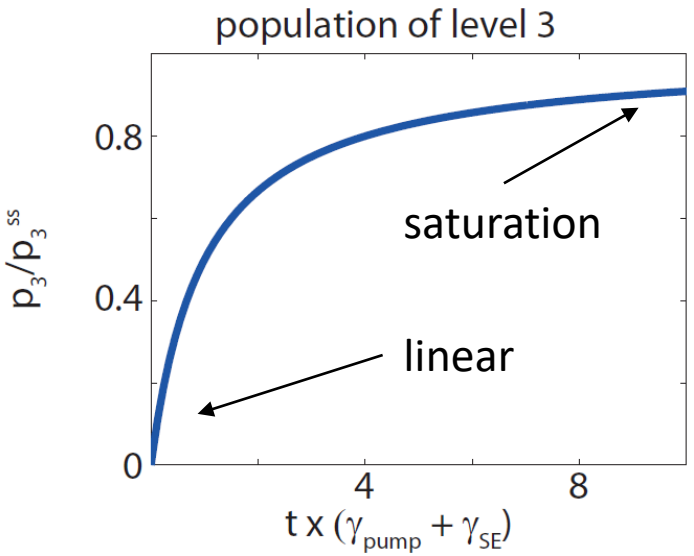
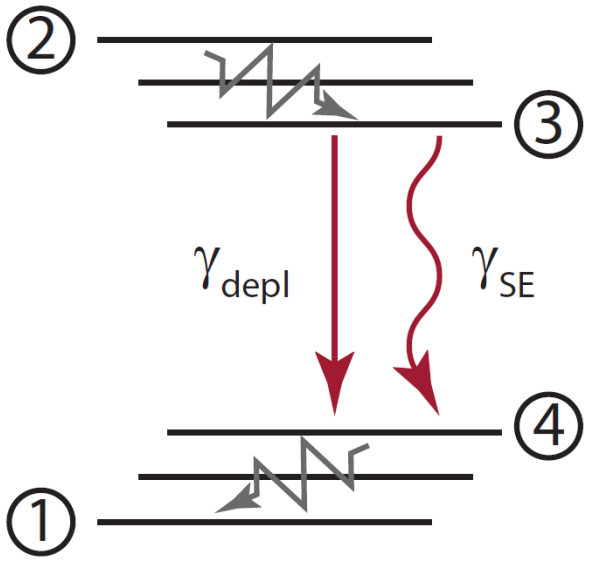




Population of excited state in absence of STED beam

- Start in excited state (with certain probability), turn on depletion laser



Population of excited state in absence of STED beam

- Start in excited state (with certain probability), turn on depletion laser
- Exponential decrease of population as function of time
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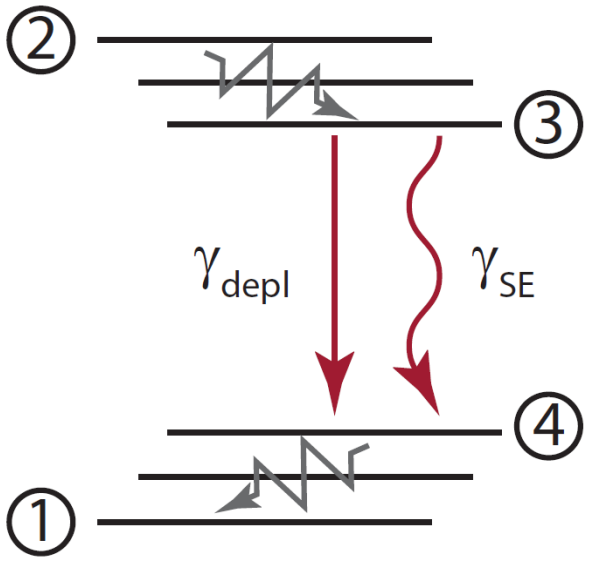

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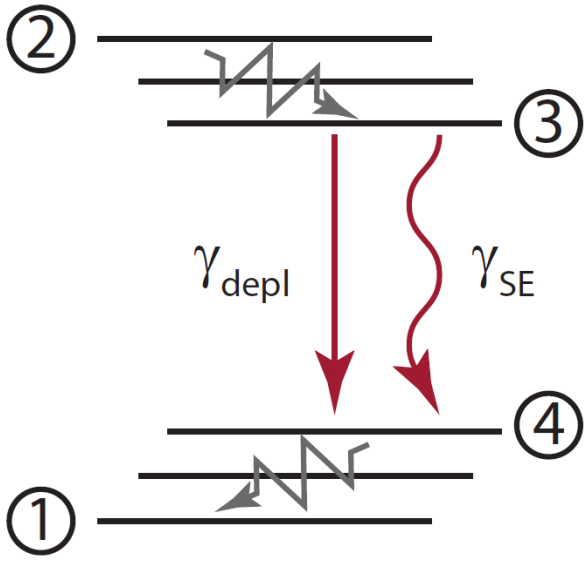
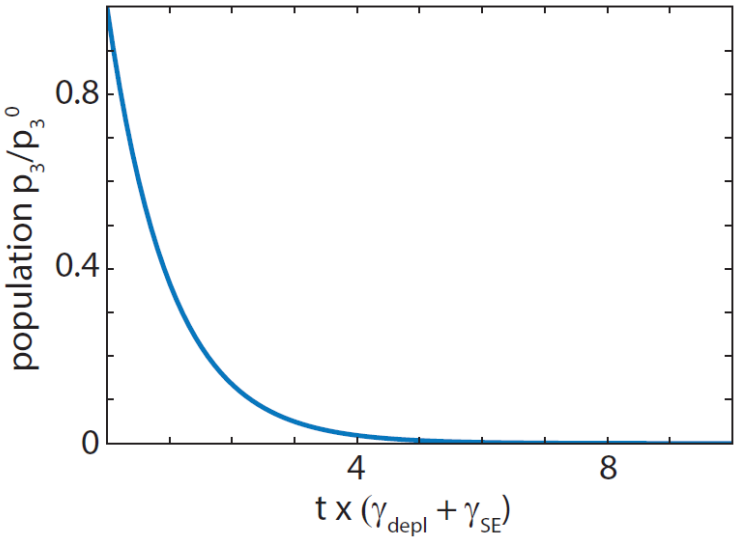
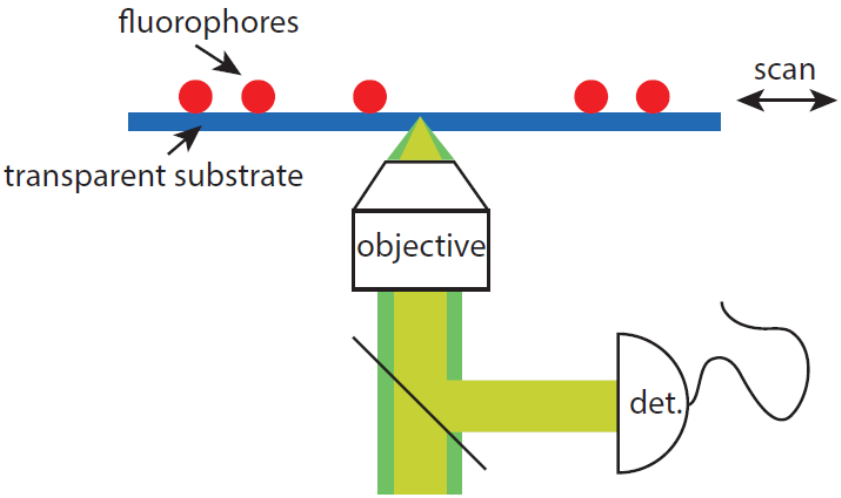
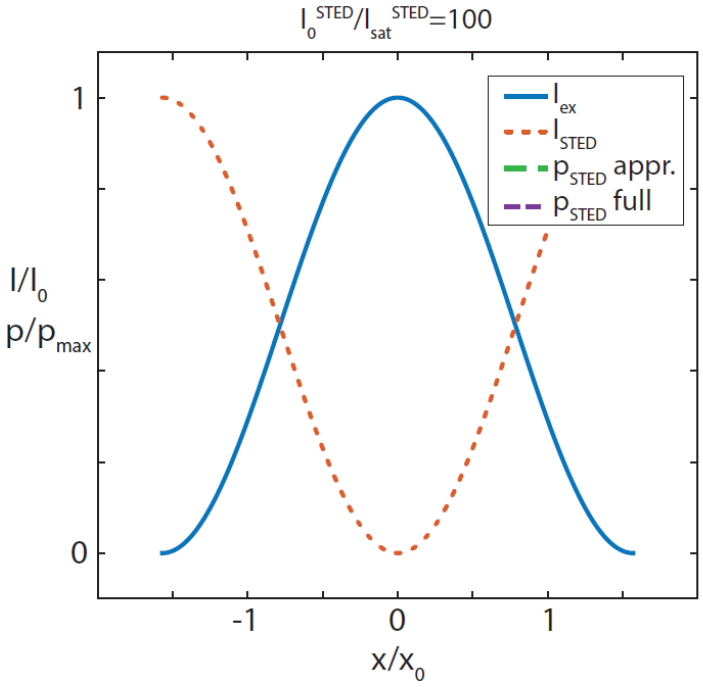




STED – how it works

- Set up overlapping excitation and depletion lasers (both can naturally only be focused to the diffraction limit!)

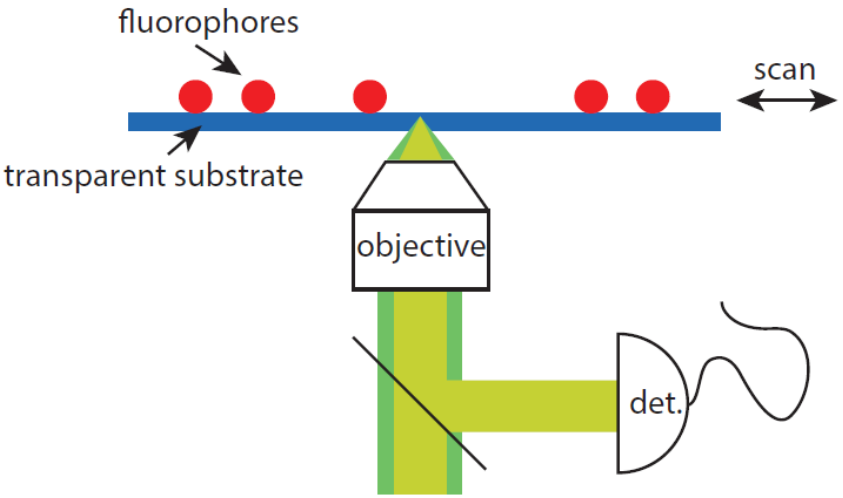


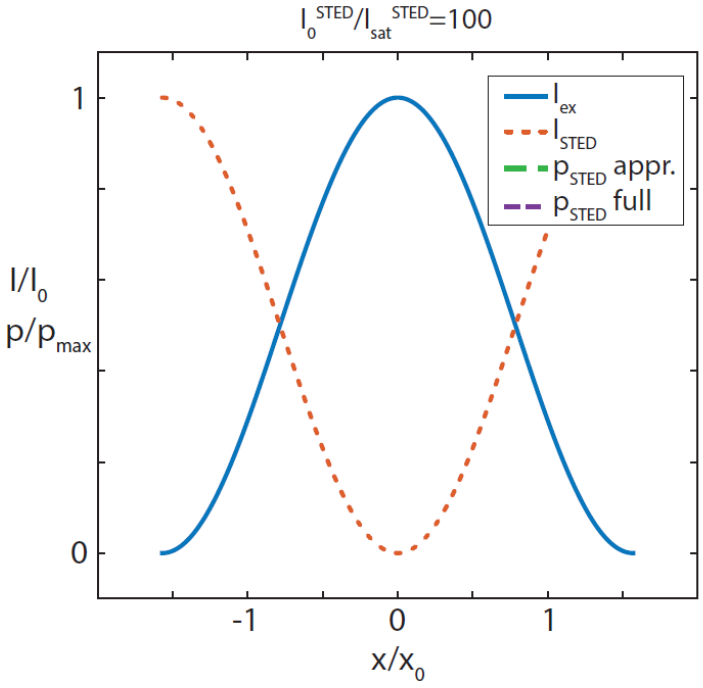


STED – how it works

- Apply a weak/short pump pulse (linear regime of charging curve)

$$p_{\mathrm{ex}}(x) = \sigma_{\mathrm{ex}}\,\tau_{\mathrm{ex}}\,I_{\mathrm{ex}}(x)/(\hbar\omega_{\mathrm{ex}})$$





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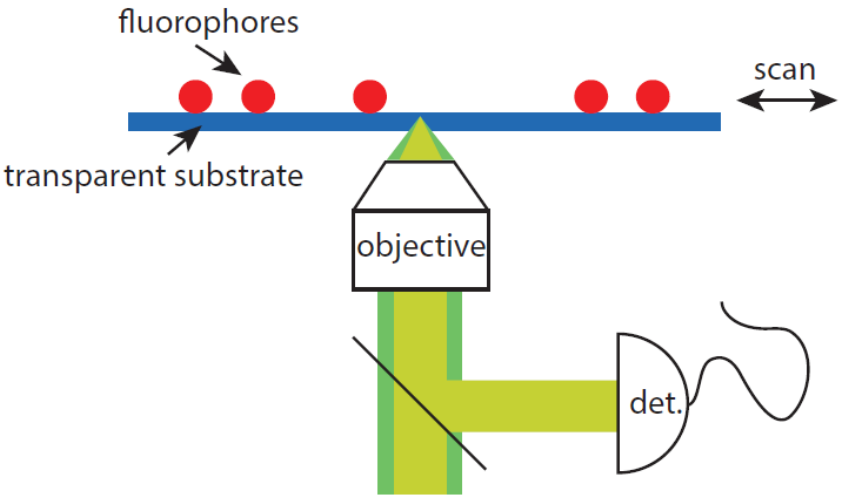
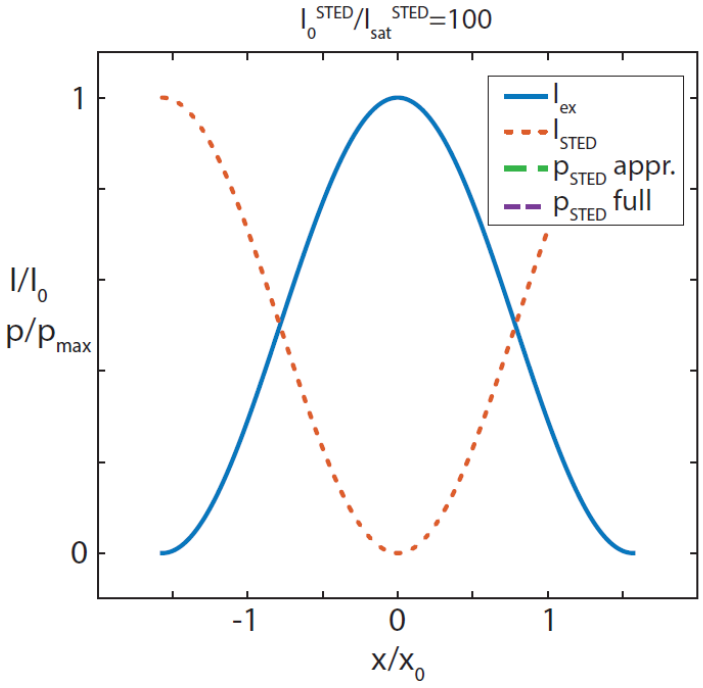
$$p_{\mathrm{STED}}(x) = p_{\mathrm{ex}}\,\exp\left[-\sigma_{\mathrm{STED}}\,\tau_{\mathrm{STED}}\,I_{\mathrm{STED}}(x)/(\hbar\omega_{\mathrm{STED}})\right]$$





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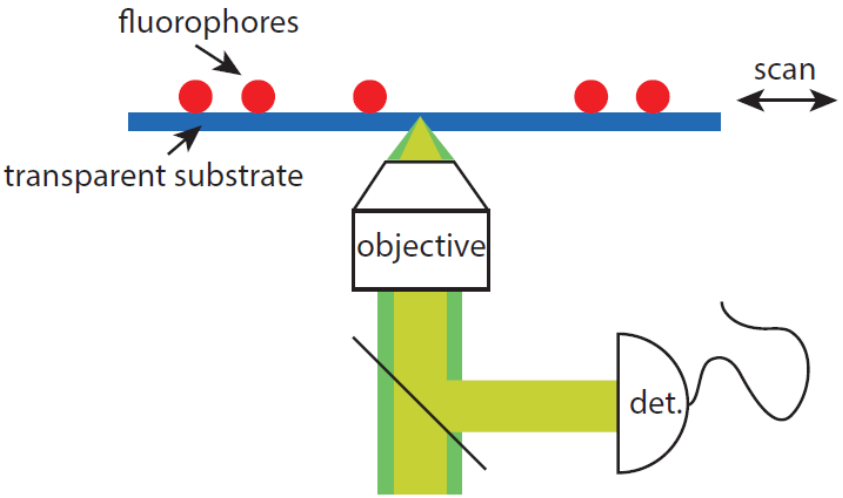
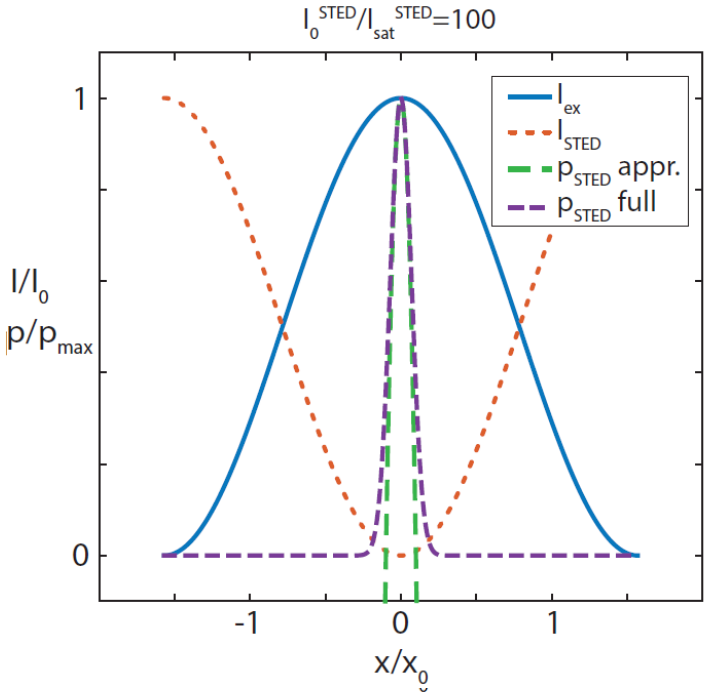
- Register fluorescence photons arriving after depletion pulse

$$p_{\mathrm{STED}}(x) \approx \frac{I_0^{\mathrm{ex}}}{I_{\mathrm{sat}}^{\mathrm{ex}}}\left[1 - \frac{x^2}{x_0^2}\left(1 + \frac{I_0^{\mathrm{STED}}}{I_{\mathrm{sat}}^{\mathrm{STED}}}\right)\right]$$





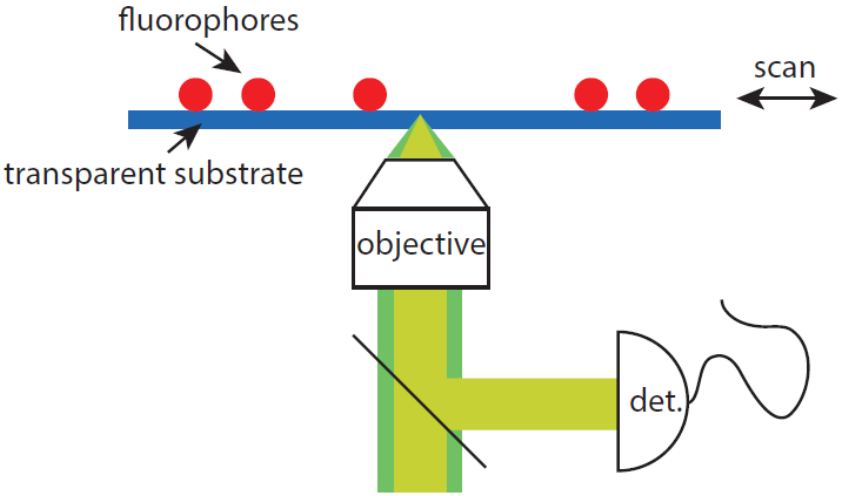
STED – how it works

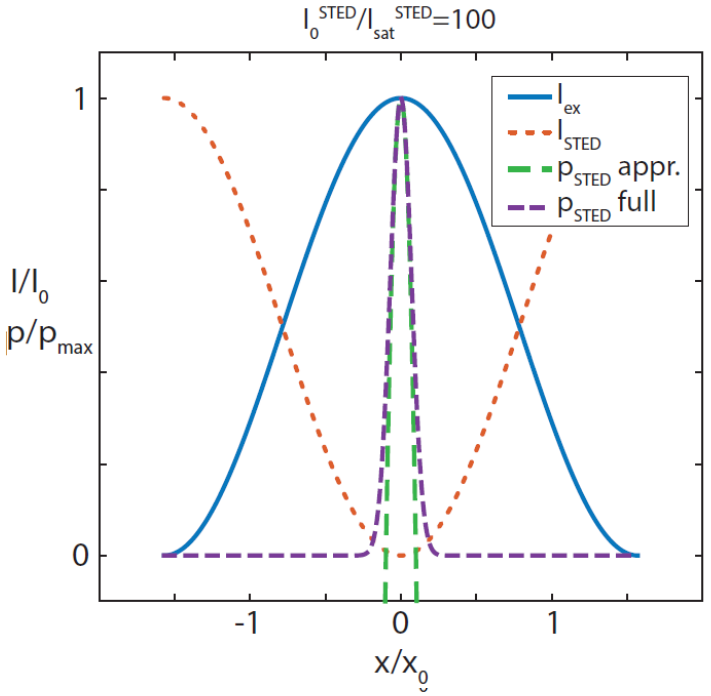
- FWHM of area of remaining pumped fluorophores after STED pulse

$$\Delta x = \frac{x_0}{\sqrt{1 + \frac{I_0^{\mathrm{STED}}}{I_{\mathrm{sat}}^{\mathrm{STED}}}}}$$

Characteristic saturation intensity:

$$I_{\mathrm{sat}}^{\mathrm{STED}} = \hbar\omega_{\mathrm{STED}} / (\sigma_{\mathrm{STED}} \tau_{\mathrm{STED}})$$





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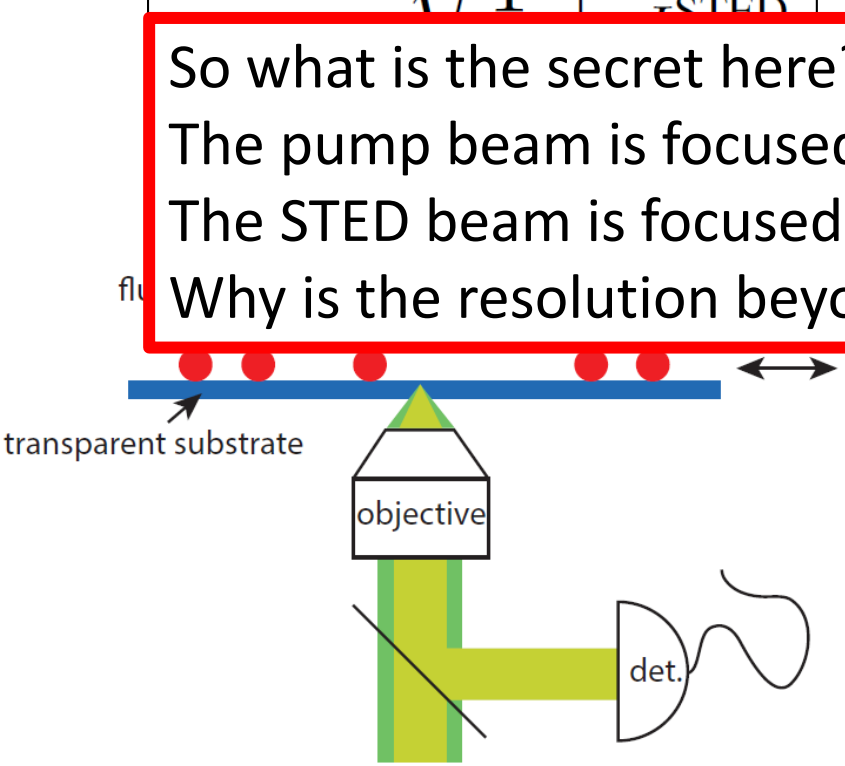
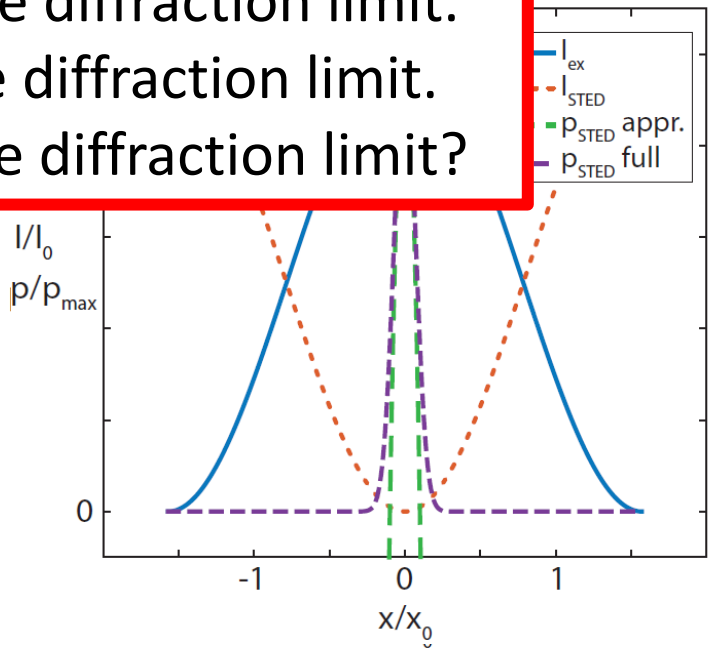
So what is the secret here?
The pump beam is focused to the diffraction limit.
The STED beam is focused to the diffraction limit.
Why is the resolution beyond the diffraction limit?

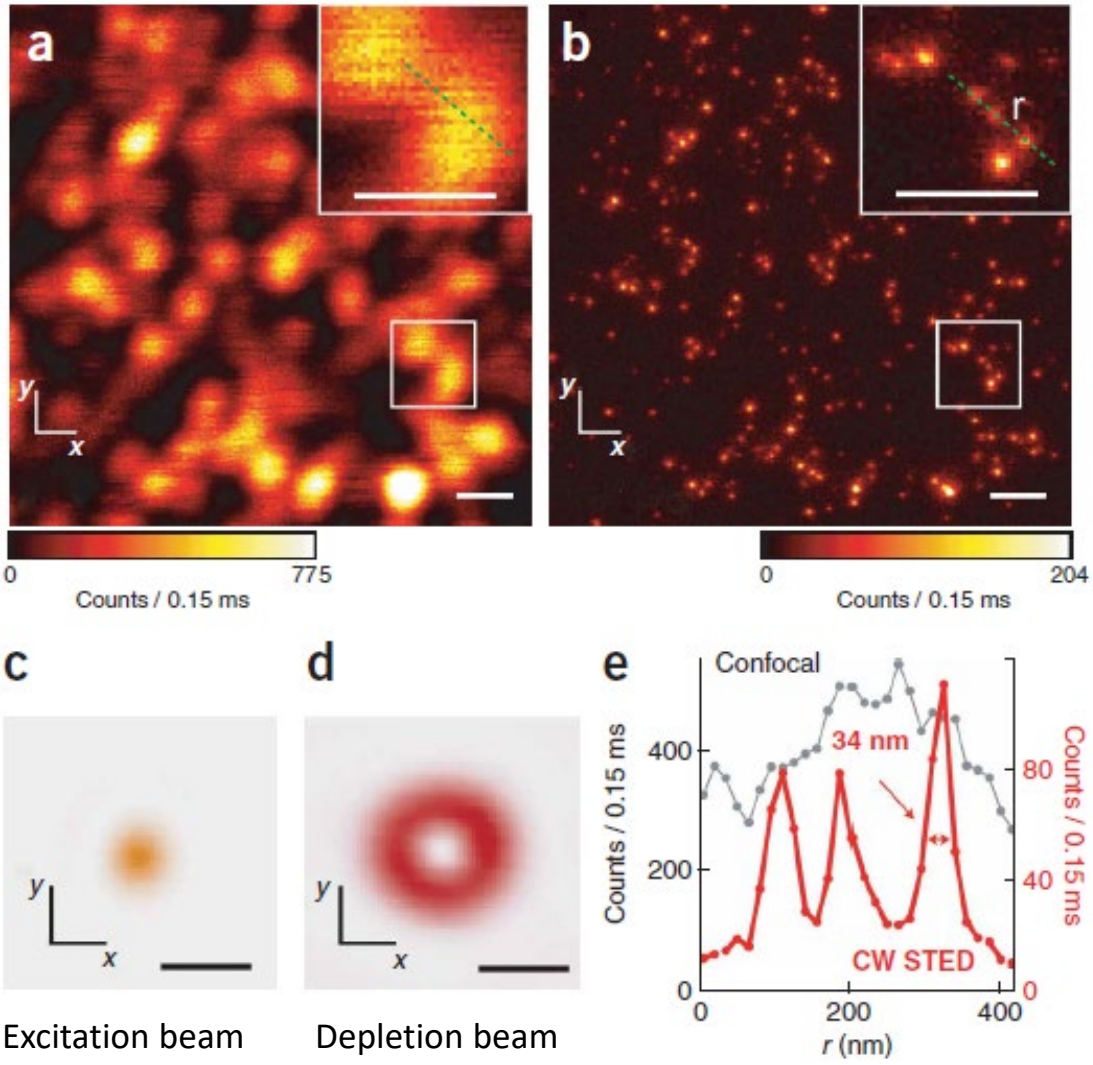




STED – how it really works

Willig et al., Nat. Meth. 4, 915(2007)

Figure 2 | Nanoscale imaging with CW STED. (**a**,**b**) Raw data of confocal (**a**) and corresponding CW-STED (**b**) image of fluorescent 20-nm-diameter beads. The images were recorded simultaneously with an excitation power of 11 μW (at 635 nm) at the sample and by turning the STED laser (825 mW, 730 nm) on and off line by line. Insets, magnification of the boxed area. Scale bars, 500 nm. (**c**,**d**) The measured focal spot of the excitation light (**c**) along with the measured focal STED doughnut exhibiting a minimum of 250 nm (FWHM; **d**). (**e**) The profile along the dashed line in **a** and **b** exhibits a spot size of 34 nm, indicating an effective resolution of ~29 nm.



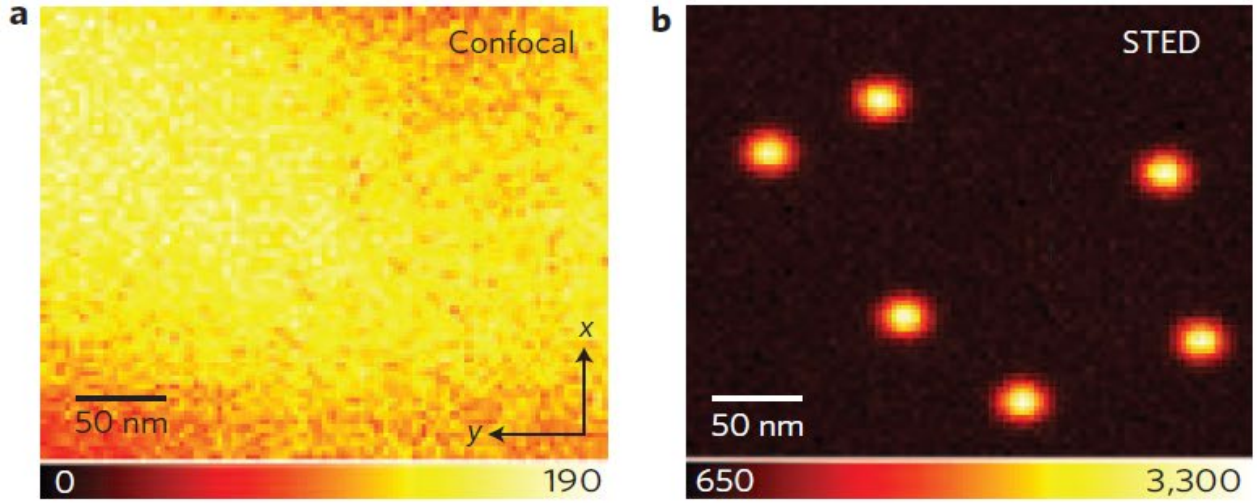
Excitation beam profile

Depletion beam profile

STED microscopy - example

Rittweger et al., Nat. Photonics 3, 144 - 147 (2009)

- Imaging color centers in diamond



- Why do I need laser pulses?
- Could I also do this with CW lasers?
- If yes, how?